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SOME CHRONIC EFFECTS OF DDT ON CUTTHROAT TROUT

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Don Allison, Burton J. Kallman
Oliver B. Cope, and Charles Van Valin

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UNITED STATES DEPARTMENT OF THE INTERIOR

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FISH AND WILDLIFE SERVICE

Clarence F. Pautzke, *Commissioner*

BUREAU OF SPORT FISHERIES AND WILDLIFE

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ABSTRACT

Effects on cutthroat trout (*Salmo clarki lewisi*) of periodic exposure to different levels of DDT in bath and in food were determined over a 20-month period involving one spawning cycle. High dosages produced a marked increase in mortality, probably by reducing resistance to nonspecific stressors; surviving fish in high-dosage lots were significantly larger than those in control and low-DDT lots. This effect occurred with no change in growth rate, perhaps because of removal of smaller and less vigorous fish by DDT. The number and volume of eggs produced was not reduced by DDT, but mortality among sac-fry appeared to be highest in the high-dosage lots. The reproductive potential of fish treated at high levels was reduced by high rates of mortality among adults and in sac-fry from survivors. Intermediate and low dosages of DDT generally did not measurably affect mortality, growth, or reproductive potential, indicating that a threshold level exists around 0.1 p.p.m. monthly in contact form and around 0.3 mg. DDT per kg. of fish weekly in the diet for the toxic effects of DDT. Metabolic conversion of DDT to DDE occurred to a greater relative extent in fish exposed to low dosages; at these levels, total residues of chlorinated hydrocarbon were not higher than in controls. Residues of DDD were present in most of the fish, possibly as a metabolite of DDT. These data suggest that the usual control treatment exposures to sublethal levels of DDT are unlikely to damage a population of rainbow trout.

SOME CHRONIC EFFECTS OF DDT ON CUTTHROAT TROUT

By Donald T. Allison, *Fishery Research Biologist,* Burton J. Kallman, *Chemist,* Oliver B. Cope, *Fishery Research Biologist,* and Charles Van Valin, *Chemist*

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DDT, 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane, is the most widely used chemical insecticide in the United States, partly because of its low cost, partly because of its insecticidal efficiency, and because of its relatively low acute toxicity to vertebrate animals. The literature on the insecticidal properties of this chemical is voluminous, and some data are available on its acute toxicity for mammals (Neal et al. 1946; Phillips and Gilman, 1946), birds (Coburn and Treichler, 1946), and fish (Henderson, Pickering, and Tarzwell, 1959).

The oral acute mean lethal doses reported for mammals range from 150 milligrams per kilogram of body weight in rats (Smith and Stohlman, 1944) to 500 mg./kg. in man (Negherbon, 1959). For birds the reported acute mean lethal doses extend from 60 mg./kg. in oil in the diet of quail (Coburn and Treichler, 1946) to 600 mg./kg. in gum acacia in the diet of young pheasants (DeWitt, Derby, and Mangan, 1955); the TLm (the concentration which half the population can tolerate) values listed for fish range from 0.01 part per million (in the medium) for rainbow trout to 0.1 p.p.m. for goldfish (Odum and Sumerford, 1946).

Large doses in mammals yield the neurologic syndrome of initial hyperexcitability, nervousness, eyelid twitching, progressing to severe and general twitching, giving way to tonic-clonic convulsions, followed by more prolonged twitchings and convulsions, and attended by difficulties of breathing. Abrupt culmination of the convulsive phase leaves the animal exhausted and motionless. Repeated doses may resolve to final, continuous, and severe tremor, with coma preceding death. In subacute dosage, effects such as hemoglobin decline with hypochromic anemia reflect a dietary cause due to prolonged appetite loss and inadequate food intake in the periods of tremor and convulsion. Some of the acute responses observed in fish are erratic swimming, loss of equilibrium, and hyperventilation.

The acute toxicity of DDT to vertebrates is relatively low, and acute mortality of vertebrates in the United States from exposure to DDT is probably not great, considering the large quantities of this chemical that are used each year. However, there is some concern over the possibilities that subclinical damage will occur as a result of exposure to low concentrations and not be recognized.

The Fish-Pesticide Research Laboratory of the Bureau of Sport Fisheries and Wild-

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life organized a series of experiments at the National Fish Hatchery at Jackson, Wyo., in 1960 to measure some chronic effects of the exposure of cutthroat trout, *Salmo clarki lewisi*, to DDT. Comparisons between untreated fish and fish exposed at different levels of DDT were made over a 20-month period.

The authors are indebted to several persons who assisted on the project in various ways. Joseph Mazuranich made facilities available at the National Fish Hatchery at Jackson, Wyo. John O'Donnell, Thomas Shen, Robert Kroesen, Diane Dwyer, and Robert Slater worked on residue analyses. W. R. Bridges, Austin Andrews, Joseph McCraren, and Vernon Bressler assisted in field operations and on logistic problems. Dr. Louis Locke made histopathology examinations of tissues.

BACKGROUND

The experiments began on December 8, 1960, and were designed to measure effects on cutthroat trout of DDT [1] taken through ingestion and through contact in water. Eleven lots of yearling Snake River cutthroat cultured in the National Fish Hatchery at Jackson, Wyo., were used, each lot containing 636 fish. One lot was an untreated control; five lots were exposed every 28 days to a 30-minute bath of DDT in 55-gallon drums coated with nontoxic asphalt paint, each lot at a given sublethal concentration; five lots were given DDT once a week in a pelleted diet, each lot receiving a given sublethal concentration; the fish averaged 6.5 inches in length, and were 21 months old when the experiment began. They were randomly divided into the 11 lots, each lot was divided into 3 sublots with equal numbers of fish, and the sublots were randomly distributed in 33 pens in 2 raceways. All 33 sublots received the same cultural treatment through the experiment, except that the fish given DDT baths received extra handling during the baths and some fish received extra handling during artificial spawning. Sample fish were withdrawn according to a schedule and were either frozen for chemical analysis for DDT residues or fixed in Bouin's solution for histopathological examination. Sublots were weighed periodically, and sample fish and those that died during the experiment were individually weighed, sexed, measured, and examined for gross pathology. Microhematocrit measurements were made from sample fish. Fish were spawned in early 1962 and the eggs were cultured in jars. The progeny from the various sublots and from different spawnings were developed and their identities retained.

The fish were fed a commercial dry diet with meat supplement before initiation of the experiment.[2] The basic diet during the experiment was a dry pelleted formulation having the following composition: Whitefish meal, 24 percent; wheat middlings, 24 percent; distiller's solubles, 21 percent; cottonseed meal, 15 percent; brewer's yeast, 10 percent; skim milk, 3 percent; cod-liver oil, 3 percent. To each 100 pounds of the mixture was added 2 pounds of corn sirup and 6 pounds of water and dye. The diet was supplemented with beef liver and spleen 2 days a week. The fish receiving DDT in the diet had the following amounts incorporated in the pellets every 7 days, one level for each lot: 3.0 milligrams per kilogram of body weight; 1.0 mg./kg.; 0.3 mg./kg.; 0.1 mg./kg.; and 0.03 mg./kg. (table 1).

The fish exposed to DDT by contact in baths were exposed to the following amounts, added in acetone solution, one level for each lot of fish: 1.0 part per mil-

[1] p,p'-DDT, 100-percent reference standard, Nutritional Biochemicals Corp., was used.

[2] Comment on chlorinated hydrocarbon residues in the commercial diets is given on p. 15.

lion; 0.3 p.p.m.; 0.1 p.p.m.; 0.03 p.p.m.; and 0.01 p.p.m.

Sample fish were taken in the following schedule: Immediately before the experiment began; 3, 7, 14, 27, 56, and 111 days after the experiment began; and about every 56 days thereafter (table 2). The experiment terminated after 16 samples had been taken, 612 days after the experiment began. From each sublot sampled, two fish were taken for chemical analysis, and two for pathology work.

TABLE 1.—*DDT treatments given experimental lots*

Lot	DDT treatment
Control:	
Lot I	None
	Parts per million
DDT in water bath:	
Lot II	1.0
Lot III	.3
Lot IV	.1
Lot V	.03
Lot VI	.01
	Milligrams per kilogram of body weight
DDT in feed:	
Lot VII	3.0
Lot VIII	1.0
Lot IX	.3
Lot X	.1
Lot XI	.03

TABLE 2.—*Sampling schedule*

Sample	Date	Days elapsed	Cumulative number of feedings of DDT	Cumulative number of exposures to DDT in bath
Sample 1	Dec. 6, 1960	-------	0	0
Sample 2	Dec. 11, 1960	3	1	1
Sample 3	Dec. 15, 1960	7	2	1
Sample 4	Dec. 22, 1960	14	3	1
Sample 5	Jan. 4, 1961	27	4	1
Sample 6	Feb. 2, 1961	56	9	2
Sample 7	Mar. 29, 1961	111	16	4
Sample 8	May 24, 1961	166	19	6
Sample 9	July 18, 1961	221	27	8
Sample 10	Sept. 13, 1961	278	34	10
Sample 11	Nov. 8, 1961	334	42	12
Sample 12	Jan. 4, 1962	391	49	14
Sample 13	Feb. 28, 1962	446	56	14
Sample 14	Apr. 20, 1962	497	63	14
Sample 15	June 21, 1962	559	72	16
Sample 16	Aug. 13, 1962	612	79	18

MORTALITY

During the course of the experiment, the fish were treated with nf-180 for furunculosis, sulfamerazine for furunculosis and kidney disease, formaldehyde for fungus and animal parasites, acetic acid for *Epistylis*, and malachite green for fungus after spawning. A recent analysis of the water at Jackson indicated a pH of 7.8, total dissolved solids of 209 p.p.m., hardness as $CaCO_3$ of 168 p.p.m., and a specific conductance of 340 micromhos at 25° C.

All of the fish which died during the course of the experiment were retained. Relatively small numbers were collected in a moribund condition and included for analysis.

It was general practice to record the date of death for each fish as well as its weight, standard length, sex, sexual development, and somatic condition. In this manner records were kept for approximately 3,000 dead fish during the 20-month experimental period. In a few cases some of the recorded data were incomplete or questionable and could not be used in certain phases of the analysis. This accounts for the discrepancy between the numbers used for cumulative mortality and those used for sex ratios.

The 33 sublots were located randomly in the two raceways. Among the three sublots of any given lot, mortality was usually a little higher in the sublot located at the lower end of a raceway. However, because the sublots were generally well scattered, this bias applied throughout all treatment levels. To simplify analysis, data from sublots have been combined and are presented as totals for each of the treatment lots.

The mean values used for cumulative mortality were subjected to t tests at 2-month intervals to determine whether the values for treated lots were significantly different from those of the control.

RESULTS

Cumulative Mortality

Cumulative mortality for lots exposed to DDT in bath and in food is presented graphically in figure 1. Although the mortality differs among lots, the mortality curves are very similar in general configuration. Fluctuations in mortality are reflected throughout most of the lots on about the same dates.

DDT in bath.—Absolute mortalities for lots II (1.0 p.p.m. DDT) and III (0.3 p.p.m. DDT) were almost identical, with only a slight temporal and numerical lag on the part of lot III. The cumulative mortality in lots II and III differed significantly from that of the control after the fourth month of exposure. By 6 months, mortality in lot II was significantly different from that of the control ($P<0.005$), and the same applied to lot III ($P<0.001$). About three times as many fish died in lots II and III as in the control group after 1 year of treatment until the end of the experiment.

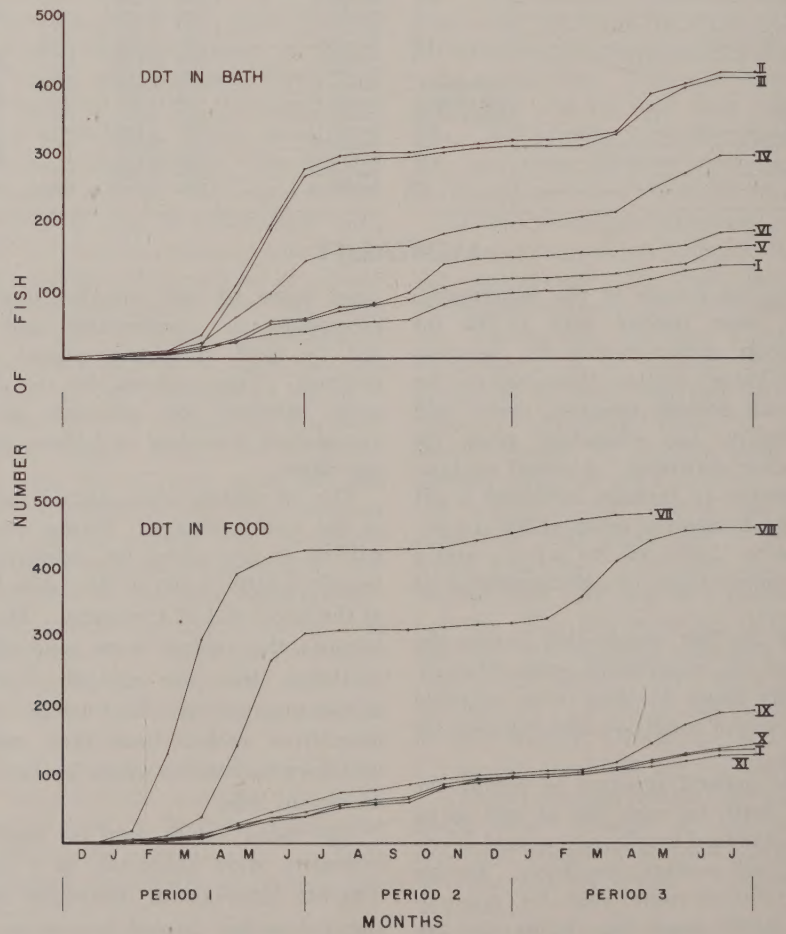

FIGURE 1.—Cumulative mortality. Each lot contained 636 fish.

Mortality in lot IV remained at a point somewhat less than halfway between the control and the two higher dosage lots throughout most of the experiment. Mortality in lot IV also differed significantly from the control after the fourth month of treatment ($P<0.005$ at 6 months) until the end of the experiment. During the last 4 months there was a marked increase in deaths in lot IV.

Differences between lots V and VI and the control were never statistically significant. However, by the end of the experiment deaths in lots V and VI exceeded those in the control by 20 percent and 37 percent, respectively. Most of this difference can be accounted for by high mortality in lots V and VI during two short periods centered about May and October 1961. These were periods of high mortality for all lots. It was found that many of the deaths in lots V and VI at these times resulted from failure of the fish to recover following release from the DDT baths. The control fish were held in aerated tanks during the administration of DDT to the bath-treated lots. Lots V and VI contained a greater total weight of fish at these times than did the other bath-treated lots. Attempts were made to keep the weight of fish per tank low enough to avoid losses from crowding. However, at the times mentioned above it appears that there were additional factors, such as reduced waterflow and handling, which caused unusual heavy losses at weight levels which had previously proved safe. As the residue levels of chlorinated hydrocarbons in samples from lots V and VI did not differ greatly from those found in the control, it is unlikely that DDT treatment was one of these factors. It is felt that these occasional excessive losses should be attributed primarily to the stress of crowding for short periods of time during treatment.

DDT in food.—Lot VII showed a sudden and marked reaction to treatment after only 1 month, and cumulative mortality in this lot differed significantly from the control at the end of 3 months ($P<0.05$). Most of the fish from lot VII which are included in the mortality data had died by the end of 7 months. At this time, mortality in this lot was more than 10 times that in the control. At the end of the experiment total mortality was still about 3½ times as great as in the control. All of the fish in lot VII not removed alive for samples had succumbed by April 1962, 3 months before the treatment of other lots was terminated.

Lot VIII evinced rapid mortality similar to lot VII about 3 months after treatment was begun, and its cumulative mortality differed significantly from that of the control following the fourth month ($P<0.01$). Mortality in lot VIII diminished at the end of the seventh month when it approached about three-quarters of the magnitude of that in lot VII. At this time, cumulative mortality in lot VII was significantly higher than that in lot VIII ($P<0.01$). However, subsequent mortality in lot VIII during the 15th through the 18th months brought total mortality for this group very close to the near total loss of fish exhibited by lot VII at this time.

Mortality in lots IX, X, and XI was almost identical to that in the control group throughout most of the experiment. Even the terminal value of lot IX was not significantly different from the control in spite of an appreciable increase in mortality during the 17th, 18th, and 19th months.

Relative mortality

Relative mortality within lots was calculated by comparing deaths per month with the average number of living fish in each lot during the month. The rates of mortality per month are expressed as percentages in figures 2 and 3. The

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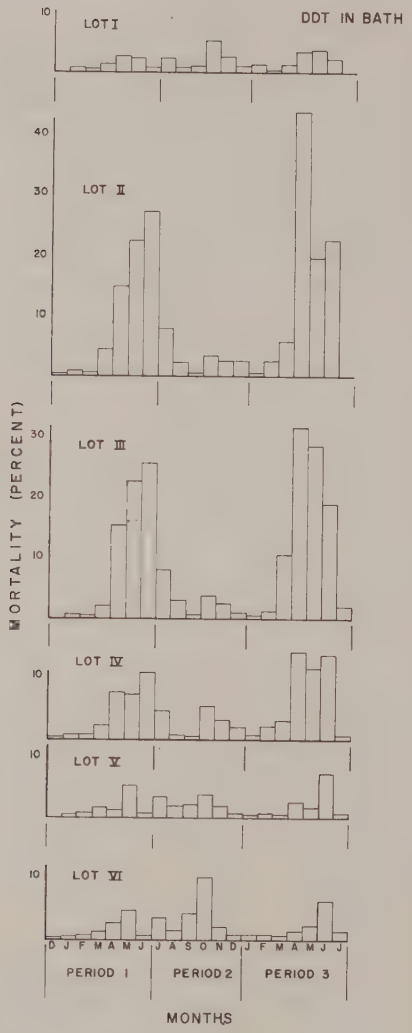

FIGURE 2.—Monthly mortality of fish receiving DDT in bath.

heavily dosed fish still had a high relative mortality toward the end of the experiment.

DDT in bath.—All of the bath-treated lots, as well as the control, displayed a trimodal distribution of relative mortality (fig. 2). These modes all fell near the 6th, 11th, and 18th months following initial treatment. The magnitude of relative mortality rates of lots II and III was similar. The highest dosage lots, II and III, also resembled each other in the low middle mode. The lower dosage lots and the control exhibited significant relative mortalities during this middle period. Mortality in lot IV paralleled that in lots II and III. Lots V and VI did not differ greatly from the control. Lot VI and the control both had their period of maximum relative mortality during the 11th month.

DDT in food.—Relative mortality rates in lot VII had a bimodal distribution in time (fig. 3). The first and higher mode occurred around the fourth and fifth months. A period of low relative mortality encompassed the 4th through 11th months. This was followed by high relative mortality until all of these fish had either died or been removed as samples by the latter part of the 17th month.

Mortality in lot VIII resembled the bimodal distribution of lot VII but occurred after a lag of 1 or 2 months. However, in this case, the second mode was greater than the first in magnitude and it closely approached the death rate of the first mode of lot VII during the previous year. As with the control and the bath-exposed fish, distribution of relative mortality rates in lots IX, X, and XI was trimodal. The first and last modes of lot IX were higher than the middle mode. Lot IX also reflected the conditions of lot VIII by displaying its highest mortality during the last few months of the experiment. The monthly death rates of lots X and XI were very similar to those of the control during the entire experiment.

Symptoms of disease

All fish which died were examined macroscopically for symptoms of disease and traumatic injury. Numbers and percentages of dead fish displaying obvious symptoms of disease at time of death (table 3) exhibited a trimodal distribution

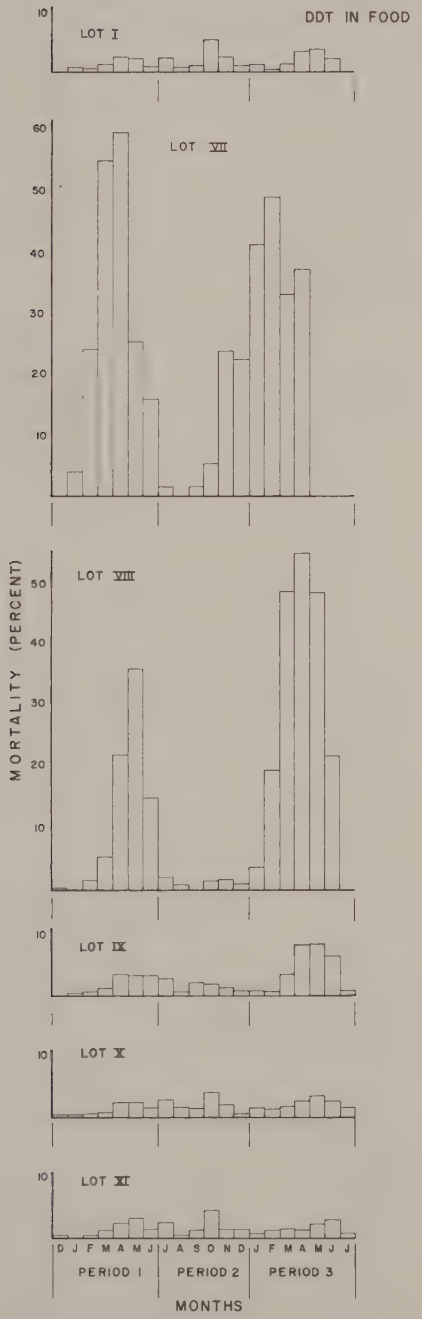

FIGURE 3.—Monthly mortality of fish receiving DDT in food

TABLE 3.—*Disease symptoms among dead fish*

Lot, treatment, and period [1]	Number of dead fish	Number of dead fish with symptoms of disease	Incidence of disease symptoms among dead fish
			Percent
Control:			
Lot I (no DDT):			
Period 1	34	21	62
Period 2	48	17	35
Period 3	41	21	51
Lot I	123	59	48
DDT in bath:			
Lot II (1.0 p.p.m.):			
Period 1	266	54	20
Period 2	42	13	31
Period 3	94	15	16
Lot II	402	82	20
Lot III (0.3 p.p.m.):			
Period 1	259	64	25
Period 2	42	4	10
Period 3	102	13	13
Lot III	403	81	20
Lot IV (0.1 p.p.m.):			
Period 1	131	34	26
Period 2	55	13	24
Period 3	98	19	19
Lot IV	284	66	23
Lot V (0.03 p.p.m.):			
Period 1	48	28	58
Period 2	61	11	18
Period 3	46	18	39
Lot V	155	67	37
Lot VI (0.01 p.p.m.):			
Period 1	49	24	49
Period 2	88	32	36
Period 3	35	16	46
Lot VI	172	72	42
DDT in food:			
Lot VII 3.0 mg./kg.):			
Period 1	396	60	15
Period 2	26	7	27
Period 3	27	5	18
Lot VII	449	72	16
Lot VIII (1.0 mg./kg.):			
Period 1	295	72	24
Period 2	14	2	14
Period 3	138	16	12
Lot VIII	447	90	20
Lot IX (0.3 mg./kg.):			
Period 1	55	25	45
Period 2	41	19	46
Period 3	86	21	24
Lot IX	182	65	36
Lot X (0.1 mg./kg.):			
Period 1	31	22	71
Period 2	53	22	42
Period 3	50	22	44
Lot X	134	66	49
Lot XI (0.03 mg./kg.):			
Period 1	42	30	71
Period 2	45	15	33
Period 3	35	16	46
Lot XI	122	61	50

[1] Period 1, Dec. 8, 1960, through June 30, 1961; period 2, July 1, 1961, through Dec. 31, 1961; period 3, Jan. 1, 1962, through July 31, 1962.

of relative mortality previously discussed. The relative incidence of disease symptoms among dead fish was much higher in the control and low-dosage lots than in the high-dosage groups (table 3). Conversely, the absolute number of disease-marked fish was highest in the high-dosage lots.

Sex ratios of mortalities

The data for the sex ratios of dead fish are given in table 4. In lots II, VII, and VIII nearly all of the fish had either died or been removed as samples by the end of the experiment, and their sex had been determined. The initial distribution of the sexes was fairly even in these three lots, and there is no reason to believe that this was not the case in the remaining lots.

In all lots except lot V, more males than females died during the first 7 months of the experiment. There was no evidence of selectivity due to differences in treatment levels. After some of the fish had died, as in the high-dosage lots, females would have outnumbered the males among the survivors. This must be kept in mind when examining the sex ratio data from the remaining periods. Since mortality of males in the control lot was accelerated to about the same degree as that of the treated fish, it appears that the toxicity of DDT was not selective for either sex.

Mortality among artificially spawned females

A record was kept during part of the 1962 spawning season on the number of females stripped of eggs in each lot and the number of these which died within the next few weeks. This information is presented in table 5.

Fish spawned from the high-dosage lots had larger average sizes and appeared to be in better physical condition than many of those spawned from the control and low-dosage lots. In spite of this, losses

TABLE 4.—*Sex ratios in fish dying during experiments*

Lot treatment, and period [1]	Number		Percent	
	Males	Females	Males	Females
Control:				
Lot I (no DDT):				
Period 1	22	12	65	35
Period 2	29	19	60	40
Period 3	23	18	56	44
Lot I	74	49	60	40
DDT in bath:				
Lot II (1.0 p.p.m.):				
Period 1	162	104	61	39
Period 2	16	26	38	62
Period 3	44	50	47	53
Lot II	222	180	55	45
Lot III (0.3 p.p.m.):				
Period 1	164	95	63	37
Period 2	16	26	38	62
Period 3	44	58	43	57
Lot III	224	179	56	44
Lot IV (0.1 p.p.m.):				
Period 1	82	49	63	37
Period 2	30	25	55	45
Period 3	49	49	50	50
Lot IV	161	123	57	43
Lot V (0.03 p.p.m.):				
Period 1	20	28	42	58
Period 2	33	28	54	46
Period 3	14	32	30	70
Lot V	67	88	43	57
Lot VI (0.01 p.p.m.):				
Period 1	30	19	61	39
Period 2	49	39	56	44
Period 3	12	23	34	66
Lot VI	91	81	53	47
DDT in food:				
Lot VII (3.0 mg./kg.):				
Period 1	222	174	56	44
Period 2	8	18	31	69
Period 3	10	17	37	63
Lot VII	240	209	53	47
Lot VIII (1.0 mg./kg.):				
Period 1	186	109	63	37
Period 2	5	9	36	64
Period 3	55	83	40	60
Lot VIII	246	201	55	45
Lot IX (0.3 mg./kg.):				
Period 1	34	21	62	38
Period 2	23	18	56	44
Period 3	54	32	63	37
Lot IX	111	71	61	39
Lot X (0.1 mg./kg.):				
Period 1	18	13	58	42
Period 2	31	22	58	42
Period 3	17	33	34	66
Lot X	66	68	49	51
Lot XI (0.03 mg./kg.):				
Period 1	27	15	64	36
Period 2	29	16	64	36
Period 3	12	23	34	66
Lot XI	68	54	56	44

[1] Period 1, Dec. 8, 1960, through June 30, 1961; period 2, July 1, 1961, through Dec. 31, 1961; period 3, Jan. 1, 1962, through July 31, 1962.

following spawning were higher in stripped females from the high-dosage lots than from the low-dosage lots. The death rates of spawned females in the high-dosage lots were considerably higher than the death rates for the entire groups.

TABLE 5.—*Mortality among spawned females compared with mortality among remaining fish and with mortality for all fish*

[Data for period Feb. 21, 1962, to Apr. 30, 1962, for lots II and VIII and for period Feb. 21, 1962, to Apr. 12, 1962, for all other lots]

Lot	Treatment	Spawned females only			All fish except spawned females			All fish including spawned females		
		Number of fish	Number dying	Percent dying	Number of fish	Number dying	Percent dying	Number of fish	Number dying	Percent dying
Control: Lot I	0	39	4	10.3	333	8	2.4	372	12	3.2
DDT in bath:	*P.p.m.*									
Lot II	1.0	50	32	64.0	128	30	23.4	178	62	34.8
Lot III	.3	35	16	45.7	152	10	6.6	187	26	13.9
Lot IV	.1	37	4	10.8	236	7	3.0	273	11	4.0
Lot V	.03	39	2	5.1	331	3	.9	370	5	1.4
Lot VI	.01	39	2	5.1	284	2	.7	323	4	1.2
DDT in food:	*Mg./kg.*									
Lot VII	3.0	1	1	100.0	16	9	56.3	17	10	58.8
Lot VIII	1.0	23	20	87.0	128	76	59.4	151	96	63.6
Lot IX	.3	51	6	11.8	326	13	4.0	377	19	5.0
Lot X	.1	44	2	4.5	330	6	1.8	374	8	2.1
Lot XI	.03	33	1	3.0	319	5	1.6	352	6	1.7
All lots		391	90	23.0	2,583	169	6.5	2,974	259	8.7

DISCUSSION

All of the experimental lots, the control included, displayed peaks of mortality during spring and early summer of both years. None of the fish exhibited typical symptoms of acute DDT poisoning. However, the lots which received the most DDT had the highest death rates at these times. It can be surmised that DDT was acting in conjunction with other factors in producing mortality.

Another peak in mortality occurred in October 1961 in all lots except VII and VIII. This peak was relatively low in lots II and III. The control and lots VI, X, and XI suffered more losses than in any other month. The high-dosage lots had lower mortality rates than the control.

Some factors which may have affected mortality rates were temperature, disease, drugs administered for treatment of disease, and handling during the spawning period. Deaths sometimes followed the use of drugs, but there was no apparent correlation between such treatment and the periodic accelerations in mortality rates. Spawning in the spring of 1962 contributed to mortality; the heaviest losses were in the high-dosage lots.

Evidence of disease was prevalent among the dead fish (table 3). Throughout the experiment the low-dosage lots contained obviously sick fish which survived for extended periods of time but were the first to succumb during the periods of high mortality. Fish in the high-dosage lots displaying signs of illness seldom survived long. The second period noted in table 3 included the October 1961 peak in mortality of the control and low-dosage lots. During this period there was a low incidence of disease symptoms in all the fish, associated with low mortality rates in the high-dosage lots. The higher mortality in low-dosage and control lots during this period may reflect an additional stress factor operating on weaker fish, perhaps of a constitutional type which had largely been eliminated during the first period in the high-dosage lots. The periodicity of mortality in all lots, including the control, seems to indicate that DDT in the treated lots increased mortality in this experiment by reducing resistance to other, nonspecific, stresses.

GROWTH

Throughout most of the experiment the pens of fish were inventoried at intervals of approximately 4 weeks. The average weight of the fish in the individual sublots was used as a measure of growth and for calculation of the feeding schedule. Calculation of true growth was complicated by the removal of living fish for scheduled samples and by mortality between inventory dates. Thus, most increments in average weight between inventory dates are biased to some extent. The analysis which follows is based on apparent growth as derived from the average weight of surviving fish.

Weights from the three sublots for each treatment level have been grouped to give the average weight per lot used in the analysis. Differences between sublots within treatment levels were small. The average weights of treated lots were statistically compared to the control by *t* test throughout the experiment.

RESULTS

Apparent cumulative growth

Figure 4 illustrates the cumulative

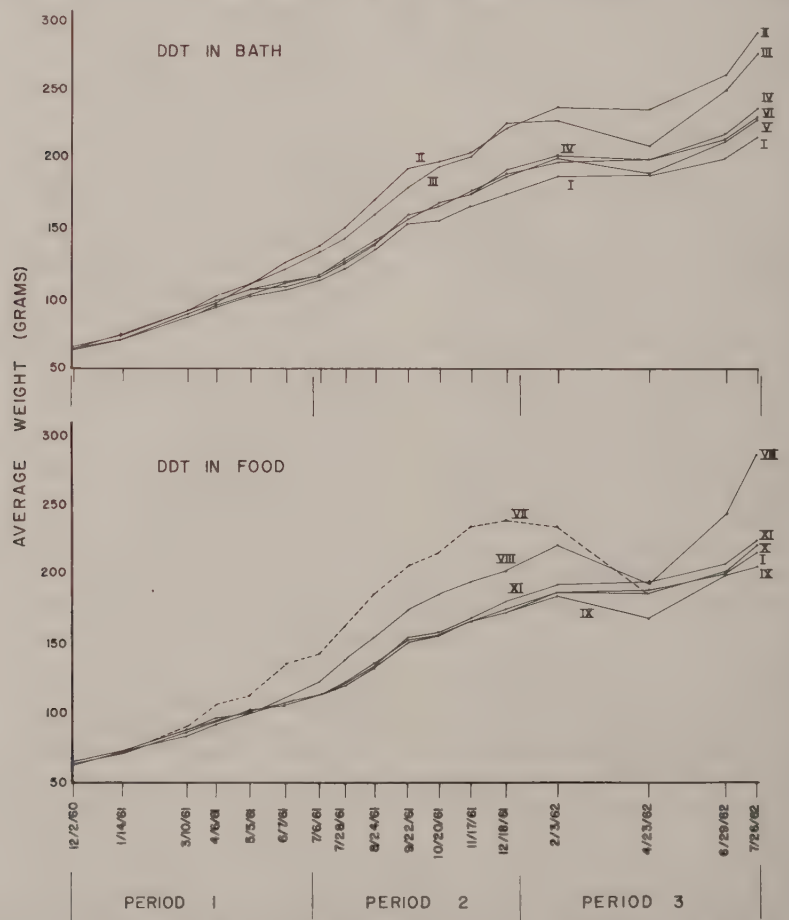

FIGURE 4.—Average weights of surviving fish. The dashed line representing the data for lot VII signifies the reduced numbers of fish present.

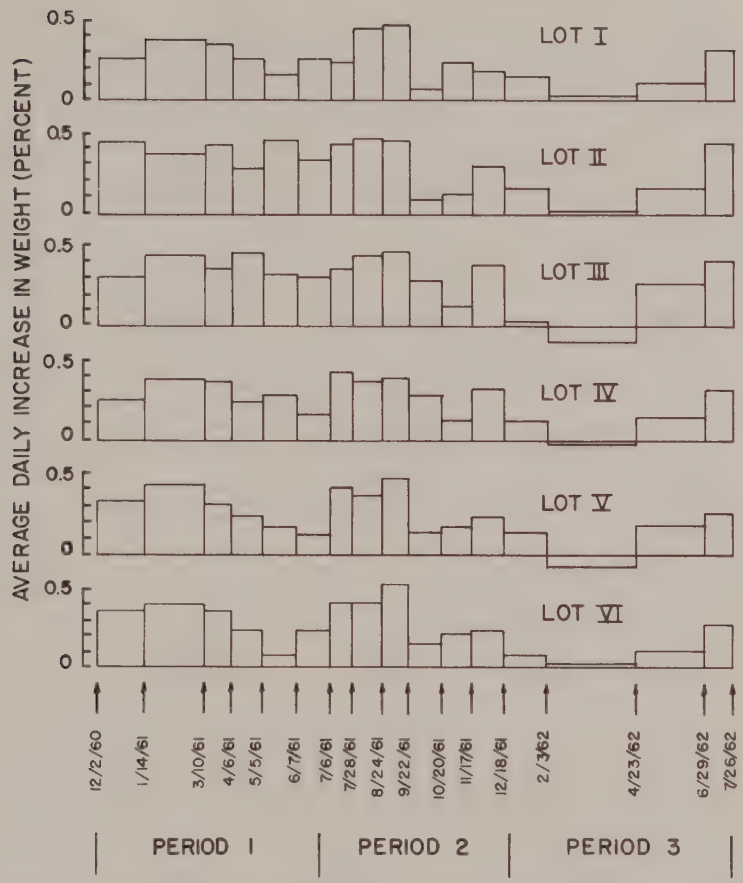

FIGURE 5.—Average daily weight changes in surviving fish receiving DDT in bath.

changes in average weight of surviving fish in each of the treatment lots. The average weights of the fish in lots II, III, VII, and VIII began to diverge from those of the other lots from February to May, and were significantly greater than the average of the control following 6 or 7 months of treatment. In statistical comparisons between these groups and the control, t values showed the differences to be significant ($P<0.025$). Values for the lower dosage groups did not generally differ significantly from the control; the few exceptions were confined to lots IV and V from December 1961 to February 1962.

Apparent growth rates

Average daily increases in weight between inventory dates, expressed as a percent of the average weight at the beginning of each period, are presented in figures 5 and 6. These values demonstrate that the apparent growth rates for short periods of time were quite similar for all treatment levels. Periodic fluctuations in apparent growth rate were reflected throughout all of the lots at approximately the same date.

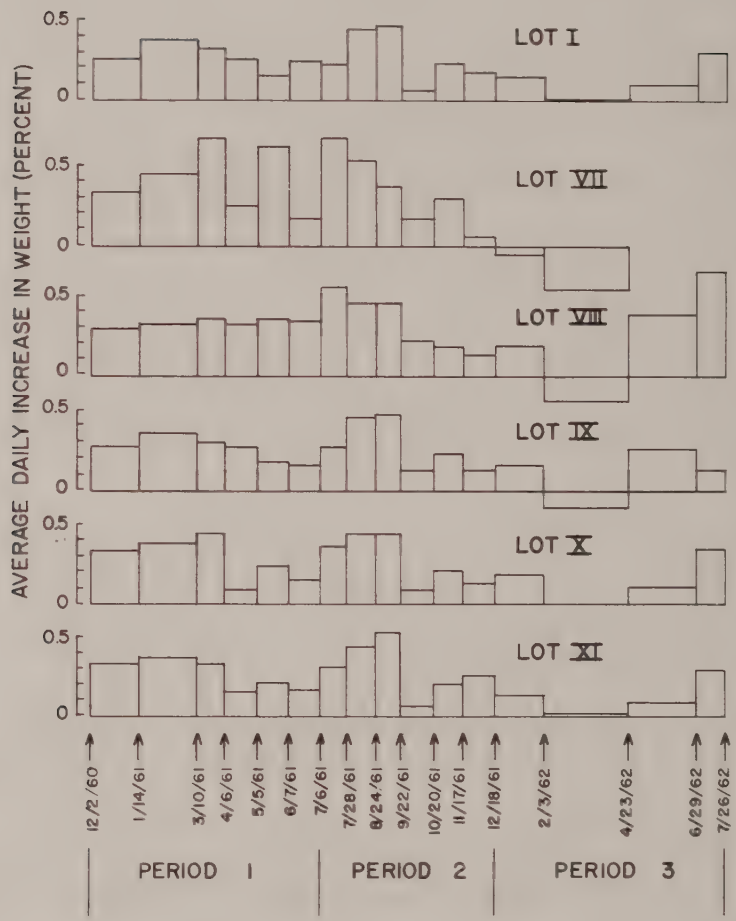

FIGURE 6.—Average daily weight changes in surviving fish receiving DDT in food.

DISCUSSION

Effects of sampling and mortality

Fish removed for scheduled samples were usually somewhat smaller than the average, and their removal tended to increase the apparent growth. However, after the initial 2 months, the numbers taken for this purpose were small. The total loss of fish from sampling was approximately the same for all lots throughout most of the experiment. Therefore, to simplify analysis, it may be assumed that bias from this source was applied equally at all treatment levels.

Weights of fish that died during the experiment were compared with the average weights of living fish at each inventory date. The samples included all dead fish collected daily for 4 days before, 4 days after, and on each inventory date. It is difficult to assess quantitatively the degree to which the high-dosage lots differed from low-dosage

and control lots, since cumulative mortalities varied so markedly. It appeared, however, that dead fish of lot I did not differ in weight from the survivors of that lot, whereas throughout the first year in all treated lots the average weight of the dead fish was to some degree lower than that of the survivors. As the populations of the treated lots became depleted, the relative incidence of large dead fish increased. During the spawning season in the spring of 1962 the dead fish averaged slightly heavier than the survivors. As most of the ripe fish in any lot were also the largest fish, the brunt of the stress from handling at this time was borne by the heaviest individuals. However, dead fish in lots VII and VIII began to outweigh the survivors as early as December 1961, long before spawning operations were started.

Effects of spawning operation

During the spring of 1962, all lots were handled frequently and, in addition, they were not fed for extended periods before being spawned artificially. The effects of handling and starvation are illustrated in figures 4, 5, and 6 for the period February 3 through April 23, 1962, when apparent growth rates ranged from small positive to large negative values. As might have been expected, the fish became thin and most of the apparent decrease in growth-rate for this period reflects the actual condition of the fish, including loss of body mass when eggs were extruded. In addition, the death of heavier-than-average fish at this time tended to reduce the apparent growth rate.

Immediately after spawning, all surviving lots showed some increase in average weight. However, mortality rates were high at this time, and growth values are considered to be strongly biased.

True growth

A sudden drop in mortality occurred after the end of June 1962. Some lots had no mortality and others lost from one to five fish. Since no samples were removed during the period June 29 through July 26, 1962, the increment of growth between these dates may be construed to approach a measure of the true growth rate at this time. The average weight and the number of fish in each lot did, however, vary considerably. All of the fish used in the experiment were the same age, but the high-dosage lots were composed predominantly of large fish which had demonstrated good growth up to this time. Therefore, it is not too surprising that these lots displayed the greatest percent increase, as shown in table 6. This suggests that growth is not inhibited in fish exposed to higher dosages of DDT.

Data for lot VII are absent from table 6. This lot was depleted through mortality and sampling some months earlier. Fortunately there was a 56-day period in the late summer of 1961 when lot VII suffered no mortality or losses due to sampling and the control was affected only slightly by these factors. As shown in table 7, percentage growth was almost identical in these lots at that time.

A search for an explanation why fish surviving exposure to the most DDT were the largest fish during most of the experiment considered the following possibilities: that it was a chance occurrence; that DDT killed the small fish, and spared the large ones; that DDT killed the diseased, slow-growing fish, and left the fast-growing ones; that DDT stimulated growth; and that a combination of the above factors was responsible.

Chance is ruled out as an explanation for the occurrence of larger fish in the heaviest treated groups. Figure 4 presents too consistent a picture of the relative positions of each group throughout the 20-month span.

Selection by the elimination of small fish in the high-DDT lots was probably

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TABLE 6.—*Increase in weight of fish during period June 29 through July 26, 1962*

Lot	Treatment	Number of fish	Initial average weight (grams)	Increase in average weight (grams)	Percent increase in weight	
					Entire lot	Range for sublots
Control:						
Lot I	0	305	201	16	8.0	6.9–8.6.
DDT in bath:	*p.p.m.*					
Lot II	1.0	50	261	31	11.9	7.3–14.4.
Lot III	.3	48	250	27	10.8	7.3–29.1.
Lot IV	.1	145	219	18	8.2	6.4–9.3.
Lot V	.03	291	214	15	7.0	6.5–8.5.
Lot VI	.01	263	215	16	7.4	3.2–9.2.
DDT in food:	*mg./kg.*					
Lot VIII	1.0	16	244	43	17.6	1 sublot.
Lot IX	.3	263	200	6	3.0	0–6.3.
Lot X	.1	307	202	19	9.4	6.6–12.6.
Lot XI	.03	291	209	17	8.1	6.0–11.6.

TABLE 7.—*Increase in weight of fish in lots I and VII during the period July 28 through September 22, 1961*

Lot	Treatment	Number of fish	Initial average weight (grams)	Increase in weight (grams)	Percent increase in weight	
					Entire lot	Range for sublots
Lot I	0	458	121	32	26.4	25.4–28.0
Lot VII	3 mg./kg	60	163	44	27.0	25.6–30.7

a factor in this occurrence, although our data did not show significant differences which could be correlated to dosage. Selection by the elimination of low-vigor, slow-growing fish by DDT might also help explain what happened. It has been pointed out in the discussion of disease and survival that fish with visible pathology were more quickly killed if they had been exposed to large amounts of DDT than if the exposure had been slight. This suggests the possibility that the survivors in high-dosage lots grew faster than those in low-dosage lots, since the low-dosage lots had a higher incidence of disease and presumably had more fish of low vigor. It is assumed that disease was originally as prevalent in large fish as in small ones. Our data on disease do not reveal any relation between fish size and incidence of disease, so this mechanism remains a possibility for explaining differences in fish size from lot to lot.

This concept appears to be compatible with the growth and mortality data. The differences between lots in growth rates would be expected to be related to differences in mortality rates. Figures 2, 3, and 4 show that size of fish in lots II and III began to diverge from that of fish in other lots in March 1961; mortality exceeded that in other lots in March. Size in lot VII began to increase over the control in February, the same month in which mortality rates became significantly different from that of the control. Lot VIII had important increases in mortality in March, and the corresponding increase in average size of surviving fish appeared in the same month. This connection between increase in mortality and differences in size lends credence to the theory that highly dosed lots of fish were larger because of the rapid death of small or diseased slow-growing individuals.

The explanation for the occurrence of large fish in the high-dosage lots may lie in the selective death of smaller and weaker fish with less growth potential.

RESIDUES

METHODS

Residues of chlorinated hydrocarbons were determined by a paper chromatographic procedure which is a modification of the method described by Mitchell (1958). The procedure as used in this laboratory has been described in detail (Kallman et al., 1962). Two purification steps were taken in preparing extracts for chromatography. These were acetonitrile partition and sulfonation. The use of MgO for cleanup was avoided, since it decomposes DDT and DDD (1,1-dichloro-2,2-bis(p-chlorophenyl)ethane), both of which were present in the fish.

Analyses were performed on aliquots of homogeneous blends of whole fish and anhydrous Na_2SO_4. In analyzing adult fish, 50 grams of tissue was present in the aliquots; when the fish became large enough, these determinations were performed in duplicate and the results were averaged. Fry samples and pooled organs or tissues were treated similarly to adult fish. It was necessary to homogenize eggs in petroleum ether:diethyl ether (9:1, v:v) in a blender; the solids were filtered off and reextracted by shaking in the same solvent mixture. A minimum of one adult fish from each sublot was analyzed at each sampling.

RESULTS

Fairly high residues were found in control fish. The commercial diet on which the fish were fed before the start of the experiment contained chlorinated hydrocarbons on the order of 0.8 p.p.m. Chlorinated hydrocarbons were present in the basic diet fed to all the fish in the experiment. The main source of this material was a grossly contaminated batch of cod-liver oil, which was replaced after having been used for about 1 year. Table 8 contains analytical data for the various components of the feed and the final mixture. Table 9 contains residue data for adult fish expressed as total chlorinated hydrocarbon in parts per million; figures 7 and 8 show these data plotted.

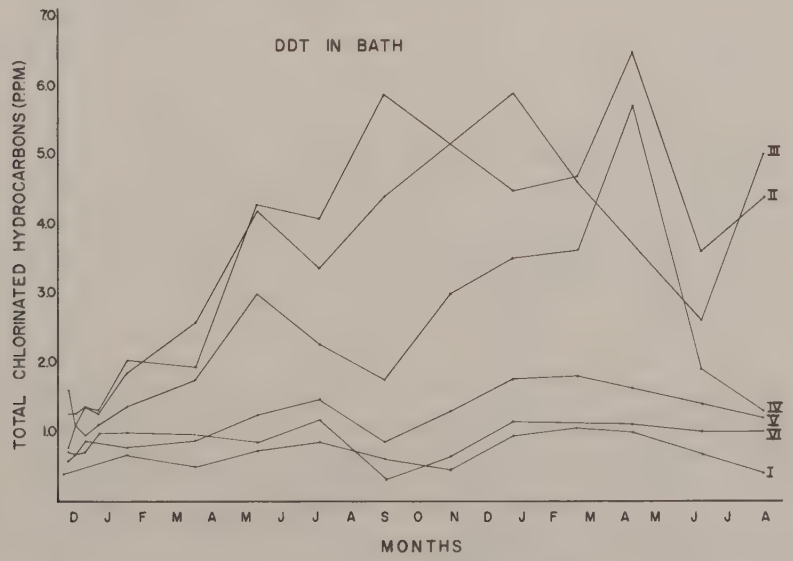

FIGURE 7.—Whole-body residues of chlorinated hydrocarbons in fish receiving DDT in bath.

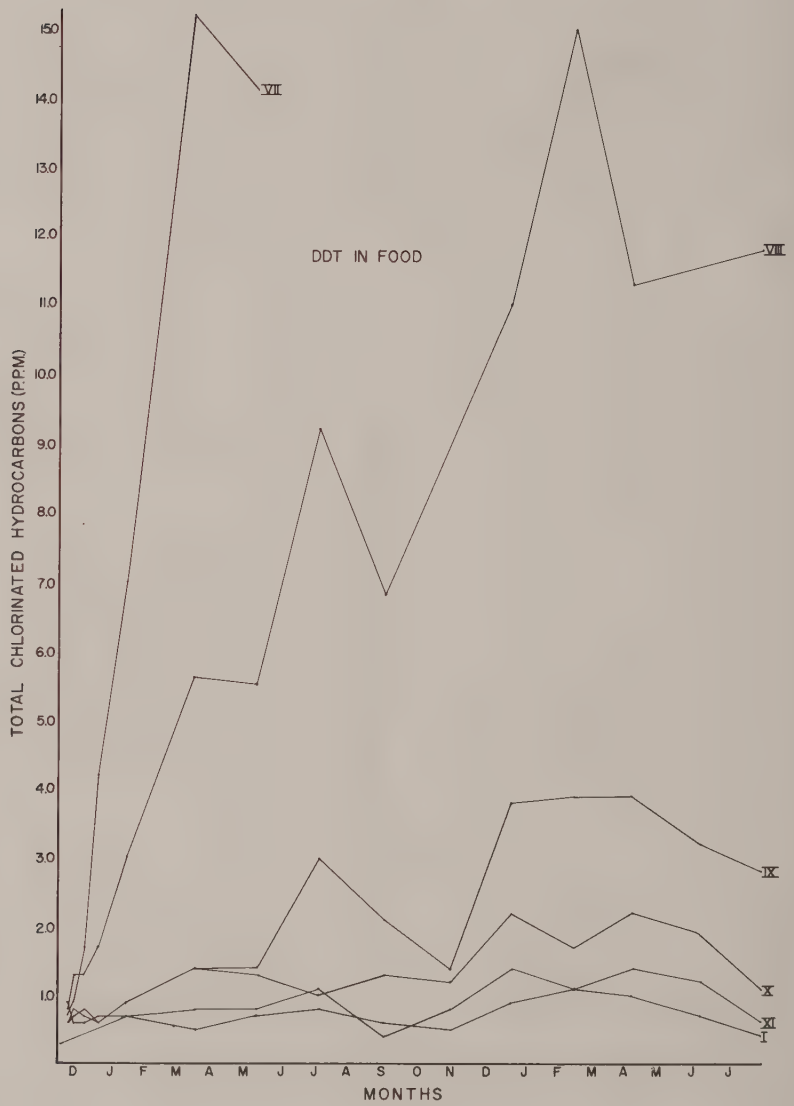

FIGURE 8.—Whole-body residues of chlorinated hydrocarbons in fish receiving DDT in food.

Residue data for eggs and fry are presented in table 10, and in table 11 for pooled organs and tissues of fish which died. The fish included in these pools died within the period November 1961 to March 1962. Table 12 shows residue data of adult fish in terms of relative amounts of the three chlorinated hydrocarbons found, DDT, DDD, and DDE (1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene).

TABLE 8.—*Concentrations of chlorinated hydrocarbons in components of the basic ration*

[Parts per million. ND=None detectable]

Component	DDT	DDE	DDD	Total
Mixture fed	0.15	0.46	0.15	0.76.
Cod-liver oil	3.0	17.0	3.7	23.7.
Molasses	ND	ND	ND	ND.
Skim milk	ND	ND	0.04	0.04.
Wheat middlings	ND	ND	Trace	Trace.
Whitefish meal	Trace	Trace	0.05	0.05+.
Yeast	Trace	Trace	Trace	Trace.
Cottonseed meal	0.03	0.04	Trace	0.07+.

DISCUSSION

Examination of the standard deviations presented in table 10 provides an indication of the extreme individual variation encountered in residue analysis for chlorinated hydrocarbons. This was found with both bath and feeding exposures. Despite this variation, definite trends can be found in the residue data. Considering the bath-treated fish, persistently high residues were found in lots II and III. These two lots did not differ significantly from each other in either mortality or residue buildup; this indicates that at concentrations of about 0.3 p.p.m. maximum uptake of DDT occurs, under the conditions of this experiment. Both lots appeared to reach a plateau of residue buildup; lot II did so somewhat earlier than lot III. Lot IV accumulated chlorinated hydrocarbon at a slower rate and continued to build up in residue throughout most of the study. Until late in the experiment, its residue amounts were distinct from those of lots receiving higher and lower treatment levels. Lot VI was almost identical to the control fish in amount of residues. Lot V was significantly different, by *t* test, from the control group at various times, but not at other times. This probably resulted from individual variation and the relatively small number of fish analyzed at each sampling; it seems extremely unlikely that this effect, or the crossing over of the residue curves of lots II and III, represents real biological events. Both exposures of 0.01 and 0.03 p.p.m. (lots VI and V) thus appeared to produce residue buildups not appreciably different from the controls, indicating an approximate equilibrium between accumulation and metabolism and excretion.

More distinct differences were observed in the lots receiving DDT in their feed than in those bathed in DDT. Residue accumulation in lots VII and VIII proceeded at rapid and different rates. There appears to be no limit to the initial rate of DDT uptake from food with the dosages used in this study, except that imposed by death. During the latter stages of the experiment, residues did level off or even drop somewhat in these two lots. A possible explanation for this might be an increased rate of metabolism and excretion. There did not appear to be a reduction in the amount of body fat in any of the DDT-exposed fish; presumably such a loss would lower the ability of the body to store chlorinated hydrocarbons. It is also possible that this apparent reduction in the rate of residue buildup occurred as a result of the heavy mortality in these two lots. Unfortunately, residue analyses were not performed routinely on samples of fish which died during the course of the experiment; possibly such fish contained significantly higher residues than the survivors, thus weighting future sampling toward lower results. Work in this laboratory has not indicated that fish which died during various LC_{50}[3] tests had higher residues of the test chlorinated hydrocarbons than did survivors.

Residues in lot IX were markedly lower than those of lot VIII, but higher than control levels. Lots X and XI were not significantly different from the controls.

No correlation was found between residue levels and either size or sex of fish in any of the lots.

[3] LC_{50} is the effective concentration at which 50 percent of the test animals die.

TABLE 9.—*Mean whole-body residues of total chlorinated hydrocarbons*

[Parts per million. Numbers in parentheses represent numbers of fish analyzed]

	Lot I (control)	DDT in bath					DDT in food				
		Lot II	Lot III	Lot IV	Lot V	Lot VI	Lot VII	Lot VIII	Lot IX	Lot X	Lot XI
Sample 1	0.4±0.2(12)										
Sample 2		1.2±0.2(3)	1.6±0.2(3)	0.8±0.1(3)	0.7±0 (3)	0.6±0 (3)	0.7±0.3(4)	0.8±0.1(3)	0.6±0.1(4)	0.6±0.1(3)	0.9±0.1(5)
Sample 3		1.2 .2(3)	1.1 .1(3)	1.1 .2(3)	.7 .2(4)	.7 .1(3)	.9 .2(3)	1.3 .4(3)	.8 .1(3)	.7 .2(3)	.6 .1(3)
Sample 4		1.4 .1(3)	1.3 .4(4)	1.0 .1(3)	.9 0 (3)	.7 .2(4)	1.7 .8(3)	1.3 .3(4)	.7 0 (3)	.8 .2(4)	.6 0 (3)
Sample 5		1.3 .1(3)	1.3 .2(4)	1.1 .4(3)		1.0 .2(3)	4.2 2.8(3)	1.7 .3(3)	.6 .3(3)	.6 .1(3)	.7 .1(3)
Sample 6	.7 .2(3)	2.0 .1(3)	1.8 .4(3)	1.4 .2(3)	.8 .1(3)	1.0 .2(3)	7.0 1.5(3)	3.0 .8(3)	.9 .4(4)	.9 0 (3)	.7 .1(3)
Sample 7	.5 .1(3)	1.9 .2(3)	2.6 .7(5)	1.8 .5(5)	.9 .1(3)	.9 .2(4)	15.2 3.5(5)	5.6 1.0(3)	1.4 .4(3)	1.4 .2(3)	.8 .2(3)
Sample 8	.7 .1(3)	4.3 .5(5)	4.2 .2(3)	3.0 .1(3)	1.2 .5(5)	.8 .1(3)	14.1 4.8(5)	5.5 2.2(6)	1.4 0 (3)	1.3 .2(3)	.8 .1(3)
Sample 9	.8 .2(6)	4.1 .5(5)	3.4 1.2(3)	2.3 .1(3)	1.5 .1(4)	1.2 .2(4)		9.2 1.7(5)	3.0 1.9(5)	1.0 .7(3)	1.1 .1(3)
Sample 10	.6 .2(6)	5.9 .6(3)	4.4 .3(3)	1.7 .5(3)	.9 .3(6)	.3 .1(6)		6.8 .6(4)	2.1 .7(3)	1.3 0 (3)	.4 .1(4)
Sample 11	.5 .2(6)			3.0 .7(4)	1.3 .7(4)	.6 .1(4)			1.4 .4(5)	1.2 .6(4)	.8 0 (4)
Sample 12	.9 .2(6)	4.5 .5(5)	5.9 .5(4)	3.5 .7(4)	1.8 .4(5)	1.2 .1(4)		11.0 3.4(6)	3.8 2.1(6)	2.2 .4(6)	1.4 .3(5)
Sample 13	1.1 .4(6)	4.7 1.2(5)	4.6 .3(4)	3.6 .2(4)	1.8 1.3(4)			15.0 2.5(4)	3.9 1.5(4)	1.7 .7(6)	1.1 .2(4)
Sample 14	1.0 .1(6)	6.5 4.7(6)	3.7 1.3(4)	5.7 .6(4)	1.6 1.1(4)	1.1 .2(4)		11.3 2.7(4)	3.9 1.8(4)	2.2 .7(4)	1.4 .5(4)
Sample 15	.7 .2(6)	3.6 1.6(6)	2.6 1.4(4)	1.9 .7(4)	1.4 .1(4)	1.0 .1(4)			3.2 1.0(4)	1.9 .4(4)	1.2 1.0(4)
Sample 16	.4 .1(6)	4.4 1.2(4)	5.0 1.0(5)	1.3 .6(4)	1.2 .2(4)	1.0 .2(4)		11.8 1.5(4)	2.8 .9(4)	1.1 .5(4)	.6 .3(4)

TABLE 10.—*Residues of total chlorinated hydrocarbons in eggs and fry*

[Parts per million]

Lot and sublot	Treatment of adults	Eggs		Fry	
		"Pick-offs" collected about 4 weeks after spawning	Unfertilized	Collected 2 to 3 weeks after hatching	Collected 5 to 6 weeks after hatching
Lot I: Sublot 2	0 *p.p.m.*	1.0	0.2	1.6	0.2
Lot II	1.0				2.2
Lot III:					
Sublot 1	.3	3.2	2.5	3.9	[1] 1.7
Sublot 3	.3	4.5	2.3	6.0	
Lot IV:					
Sublot 1	.1	7.4	1.2	2.4	[1] 1.2
Sublot 3	.1	5.2	2.9	4.2	
Lot V:					
Sublot 1	.03	.7	1.4	3.1	
Sublot 2	.03	1.8			.4
Sublot 3	.03	2.9	1.0		
Lot VI	.01				.8
Lot VIII:	*mg./kg.*				
Sublot 1	1.0	.7	11.0		
Sublot 3	1.0	5.3	19.8	13.2	
Lot IX:					
Sublot 2	.3	3.0	5.8	5.9	[1] .9
Sublot 3	.3	3.9	3.1	2.8	
Lot X:					
Sublot 2	.1	1.1	2.4	2.1	[1] .6
Sublot 2	.1	.7			
Lot XI	.03				.2

[1] These figures represent analytical results of pooled samples from 2 sublots.

TABLE 11.—*Residues of total chlorinated hydrocarbons in tissues of dead fish*

[Parts per million. Numbers of fish are approximate]

Tissue	Lot II (8 fish)				Lots V and VI combined (15 fish)				Lot VIII (8 fish)				Lots X and XI combined (15 fish)			
	DDT	DDE	DDD	Total	DDT	DDE	DDD	Total	DDT	DDE	DDD	Total	DDT	DDE	DDD	Total
Brain	6.2	6.3	1.0	13.5	ND	1.3	ND	1.3	28.0	7.0	1.0	36.0	0.5	0.5	Trace	1.0
Skeletal muscle	3.3	1.5	.3	5.1	0.4	.3	0.1	.8	7.8	2.3	.8	10.9	.3	.3	0.05	.6
Liver	1.3	1.8	2.5	5.6	.2	.8	.4	1.4	1.8	1.4	7.0	10.2	.3	.9	.4	1.6
Ovary	8.6	2.9	.8	12.2	1.4	1.8	.3	3.5	4.8	.9	.9	6.6	.8	.7	.2	1.7
Gut	1.0	2.4	1.3	4.7	Trace	1.5	.4	1.9	1.0	.8	2.5	4.3	1.5	5.5	.3	7.3
Testes	.6	1.0	.4	2.0	.3	3.5	.1	3.9	1.3	.7	.4	2.4	.1	.2	.1	.4

TABLE 12.—*Percentage of total chlorinated hydrocarbons as DDT, DDE, and DDD in whole-body residues* [mean percent; range in parentheses]

A. DDT IN BATH

Sample No.—	Lot I			Lot II			Lot III			Lot IV			Lot V			Lot VI		
	DDT	DDE	DDD	DDT	DDE	DDD	DDT	DDE	DDD	DDT	DDE	DDD	DDT	DDE	DDD	DDT	DDE	DDD
2	12	70	17	65	31	4	65	26	9	45	50	5	38	55	8	23	63	14
	(5–23)	(62–87)	(6–29)	(60–70)	(28–35)	(3–5)	(58–71)	(21–32)	(8–10)	(41–50)	(44–55)	(3–6)	(38–39)	(53–56)	(7–9)	(19–25)	(60–68)	(13–16)
3				56	36	8	49	42	8	46	47	7	34	55	11	23	65	12
				(53–60)	(33–38)	(7–9)	(46–55)	(38–46)	(7–9)	(39–50)	(42–55)	(6–8)	(26–43)	(51–59)	(6–16)	(19–28)	(64–66)	(8–16)
4				54	37	9	54	40	6	51	42	7	42	48	10	18	66	16
				(53–55)	(36–39)	(8–9)	(49–58)	(37–45)	(3–10)	(49–53)	(37–45)	(6–10)	(38–48)	(42–55)	(7–13)	(6–32)	(58–71)	(10–22)
5				48	45	7	48	45	7	44	45	12				12	80	9
				(43–50)	(43–49)	(6–8)	(43–54)	(41–50)	(5–11)	(42–45)	(42–49)	(8–13)				(11–13)	(75–81)	(6–12)
6	16	69	15	61	34	5	57	38	6	46	47	8	23	71	6	25	64	11
	(8–20)	(65–75)	(13–17)	(59–63)	(32–36)	(5)	(46–65)	(31–48)	(4–7)	(41–52)	(41–50)	(7–9)	(18–26)	(68–75)	(5–7)	(16–30)	(57–74)	(9–13)
7	19	72	9	61	36	3	67	30	3	61	35	4	27	65	8	25	65	8
	(12–29)	(60–80)	(8–10)	(55–64)	(32–42)	(2–4)	(56–76)	(21–41)	(2–3)	(52–76)	(23–44)	(1–7)	(25–29)	(59–72)	(4–12)	(18–35)	(50–74)	(8–15)
8	20	72	8	73	23	3	77	20	3	66	31	3	49	46	6	34	58	8
	(15–23)	(67–78)	(6–9)	(71–77)	(20–26)	(2–5)	(72–80)	(17–25)	(2–3)	(59–70)	(27–38)	(3–4)	(26–63)	(32–67)	(5–7)	(30–37)	(57–59)	(6–11)
9	15	74	10	72	22	5	76	23	1	60	36	3	56	38	6	40	53	7
	(7–28)	(60–82)	(7–14)	(67–75)	(19–27)	(4–6)	(65–84)	(15–34)	(1–2)	(54–66)	(28–44)	(1–5)	(53–62)	(36–43)	(2–9)	(30–45)	(45–64)	(5–10)
10	21	72	8	70	25	5	73	23	4	78	19	3	54	39	7	42	46	12
	(6–44)	(51–87)	(5–15)	(65–78)	(18–30)	(4–6)	(70–77)	(19–27)	(3–4)	(72–84)	(14–24)	(2–4)	(36–65)	(29–54)	(3–12)	(33–50)	(43–55)	(7–14)
11	13	80	7							64	34	3	54	42	4	20	74	6
	(4–20)	(73–90)	(6–8)							(51–74)	(22–47)	(2–4)	(48–60)	(36–49)	(3–4)	(15–30)	(62–80)	(5–8)
12	11	85	4	73	25	2	81	17	2	71	27	2	45	52	3	28	68	3
	(6–13)	(82–90)	(4–6)	(66–79)	(20–32)	(1–2)	(80–82)	(14–18)	(2–4)	(65–76)	(22–32)	(1–3)	(39–52)	(44–58)	(2–4)	(27–29)	(68–69)	(2–4)
13	14	81	4	71	26	3	70	27	2	68	30	2	39	55	6			
	(11–17)	(78–84)	(2–7)	(64–79)	(19–33)	(2–4)	(66–74)	(23–32)	(2–3)	(67–68)	(29–32)	(1–4)	(34–45)	(49–61)	(4–10)			
14	10	76	14	60	36	5	52	41	7	40	56	4	49	47	4	30	59	10
	(4–13)	(73–81)	(10–19)	(47–79)	(15–49)	(2–8)	(33–65)	(34–53)	(1–13)	(30–46)	(47–67)	(2–7)	(41–53)	(42–54)	(0–6)	(14–38)	(48–82)	(4–15)
15	34	59	8	62	36	2	60	40		50	50		38	48	14	30	58	12
	(12–58)	(34–88)	(0–32)	(48–72)	(24–52)	(0–6)	(48–70)	(30–52)		(38–62)	(38–62)		(33–50)	(34–57)	(11–17)	(25–36)	(47–68)	(7–17)
16	10	83	6	76	20	3	75	22	4	49	47	4	49	46	6	44	49	7
	(3–20)	(73–92)	(0–10)	(68–83)	(14–29)	(3–4)	(68–81)	(16–28)	(3–6)	(32–65)	(30–65)	(2–6)	(42–59)	(38–53)	(3–7)	(35–49)	(42–60)	(-10)

B. DDT IN FOOD

Sample No.—	Lot I			Lot VII			Lot VIII			Lot IX			Lot X			Lot XI		
	DDT	DDE	DDD	DDT	DDE	DDD	DDT	DDE	DDD	DDT	DDE	DDD	DDT	DDE	DDD	DDT	DDE	DDD
2	12 (5–23)	70 (62–87)	17 (6–29)	29 (7–50)	54 (37–71)	18 (13–27)	38 (29–52)	54 (40–64)	8 (7–10)	14 (5–23)	69 (65–76)	17 (13–23)	20 (17–22)	67 (61–72)	13 (11–17)	4 (0–4)	87 (84–89)	12 (9–12)
3				34 (30–39)	56 (54–60)	10 (6–13)	45 (33–63)	47 (31–58)	7 (6–9)	25 (23–27)	68 (66–70)	7 (7–8)	26 (22–34)	59 (51–65)	15 (14–16)	24 (23–26)	63 (62–64)	14 (13–15)
4				60 (57–64)	32 (30–36)	7 (5–10)	46 (31–55)	44 (36–45)	9 (8–11)	22 (19–24)	67 (65–69)	11 (7–16)	20 (15–30)	66 (56–74)	15 (14–16)	29 (25–32)	61 (58–65)	10 (9–10)
5				83 (77–87)	13 (10–18)	3 (2–5)	64 (59–74)	27 (18–31)	9 (8–10)	37 (37)	53 (51–55)	9 (8–11)	24 (18–32)	66 (59–71)	10 (9–11)	21 (17–27)	67 (63–69)	13 (10–15)
6	16 (8–20)	69 (65–75)	15 (13–17)	88 (86–90)	10 (8–12)	2 (1–3)	69 (58–77)	26 (19–37)	5 (4–5)	25 (7–40)	62 (43–76)	13 (8–17)	21 (14–32)	68 (56–75)	11 (9–12)	21 (18–26)	65 (58–72)	14 (11–17)
7	19 (12–29)	72 (60–80)	9 (8–10)	85 (83–88)	12 (9–14)	3 (2–4)	81 (72–87)	15 (10–24)	3 (3)	49 (45–53)	45 (43–49)	6 (5–8)	40 (34–46)	49 (43–55)	11 (11–12)	35 (30–38)	53 (46–56)	15 (14–16)
8	20 (15–23)	72 (67–78)	8 (6–9)	82 (66–91)	14 (6–31)	3 (2–4)	69 (34–84)	28 (11–63)	4 (3–6)	37 (36–40)	59 (57–61)	4 (3–6)	58 (57–60)	35 (33–36)	7 (6–8)	44 (37–51)	47 (42–53)	9 (8–10)
9	15 (7–28)	74 (60–82)	10 (7–14)				85 (83–88)	13 (9–15)	1 (0–3)	60 (52–65)	36 (31–45)	4 (3–5)	52 (42–60)	41 (29–53)	7 (5–10)	45 (43–48)	47 (42–50)	9 (6–11)
10	21 (6–44)	72 (51–87)	8 (5–15)				91 (88–94)	6 (4–8)	3 (2–4)	82 (80–85)	14 (12–15)	4 (3–5)	63 (62–63)	33 (32–34)	4 (3–5)	51 (37–70)	39 (11–58)	10 (3–19)
11	13 (4–20)	80 (73–90)	7 (6–8)							59 (47–74)	39 (24–49)	2 (1–4)	45 (36–57)	52 (41–59)	3 (3–4)	36 (30–40)	58 (55–65)	5 (4–5)
12	11 (6–13)	85 (82–90)	4 (4–6)				90 (89–91)	9 (8–10)	1 (1–2)	60 (27–74)	36 (23–60)	4 (2–13)	56 (50–60)	41 (37–47)	3 (2–3)	29 (19–43)	67 (51–76)	4 (3–6)
13	14 (11–17)	81 (78–84)	4 (2–7)				86 (83–90)	11 (8–15)	2 (1–5)	68 (54–77)	29 (21–43)	3 (2–5)	53 (24–67)	44 (29–74)	2 (2–4)	31 (18–40)	62 (52–77)	6 (4–9)
14	10 (4–13)	76 (73–81)	14 (10–19)				74 (67–80)	22 (14–32)	4 (1–6)	60 (43–78)	35 (18–51)	5 (4–7)	55 (51–63)	38 (30–43)	7 (6–9)	39 (27–48)	57 (51–67)	4 (2–6)
15	34 (12–58)	59 (34–88)	8 (0–32)							60 (55–65)	30 (28–35)	10 (6–11)	53 (46–62)	40 (33–47)	6 (5–8)	40 (21–66)	57 (31–79)	4 (0–10)
16	10 (3–20)	83 (73–92)	6 (0–10)				80 (77–82)	17 (14–20)	2 (2–3)	65 (58–76)	31 (20–40)	3 (2–4)	42 (24–62)	53 (33–70)	5 (4–6)	23 (17–26)	70 (65–83)	6 (0–11)

REPRODUCTION

METHODS

During the spring of 1962, about 15 months after the experiment was begun, many of the fish approached sexual maturity. Eggs were stripped from females as they became ripe through a period of more than 3 months. While lack of sufficient hatching facilities prevented the incubation of all of the eggs, an attempt was made to rear at least three collections from each of the three sublots representing each treatment level. In some cases, where few eggs were taken each time, as many as six collections were incubated from a single sublot. Eggs were fertilized with milt from males in the same sublot. All collections were kept segregated by sublot and date of spawning.

For each collection (i.e., eggs from one sublot taken on a given day) the volume of eggs was determined by displacement. The average size of the eggs in each collection was determined by sample count in a standard measuring trough.

After measurement for egg production, coagulated eggs were removed and measured to determine initial mortality. The remaining eggs were then incubated in hatching jars modified as outlined by Buss (1959). Coagulated and undeveloped eggs were removed and measured volumetrically following eyeing and hatching.

The surviving fry were raised to various stages of development in the hatching jars. Most of the fry were sacrificed for samples a few weeks after hatching. However, some of them representing most of the treatment levels were still alive and growing well more than 8 months after the eggs were fertilized.

RESULTS

Sexual development of adults

The proportion of sexually mature fish was higher in the high-dosage lots than in the low-dosage groups. Approximate percentages of females which were ripe when fish were spawned were as follows: I, 4; II, 7; III, 8; IV, 6; V, 4; VII, 10; VIII, 7; IX, 5; X, 3; XI, 4. This was apparently due to the higher incidence of small fish in the control and low-dosage lots. Subjective observation at time of spawning revealed no discernible differences in sexual development between fish of like size at any of the treatment levels.

Egg production

In all lots there were wide variations in both the size and the number of eggs produced by individual females. When these data were grouped by lot, the average egg production was similar for most treatment levels (table 13). The volume and numbers of eggs produced by lot VIII fish stands out higher than the rest, but it, along with lot III, contained larger-than-average females at this time. Moreover, the volume of eggs per unit weight of female was also highest in lots III and VIII. In any event, prolonged treatment with DDT at the higher levels apparently was not detrimental to numbers and volumes of eggs produced in the survivors.

Development of progeny

Mortality rates for developing eggs are presented in table 14. Considering the environmental factors involved, the survival rates for all lots during incubation were uniform. The notable exception was lot VII, the fish fed the most DDT, where only one female survived for spawning. Low survival in this case may have been caused by DDT or may simply reflect poor fertilization or some individual deficiency in one of the parents.

Mortality among the sac-fry of all lots was so high that it proved impractical to make accurate measurements of losses at this stage. However, the mortality of

TABLE 13.—*Egg production of artificially spawned fish*

	Treatment	A—Group of females				B—Random sample from A		
		Number of females	Milliliters of eggs per female	Estimated number of eggs per female [1]	Number of eggs per milliliter	Number of females	Average weight in grams	Milliliters of eggs per kilogram of fish
Lot I	0	45	30.7	870	28.3	15	200	172
	p.p.m.							
Lot II	1.0	52	35.8	1,000	27.8	35	201	179
Lot III	.3	37	38.1	1,030	27.1	18	221	206
Lot IV	.1	40	34.0	940	27.7	21	214	175
Lot V	.03	46	33.8	930	27.5	19	214	165
Lot VI	.01	41	33.4	880	26.2	24	201	178
	mg./kg.							
Lot VII	3.0	1	32.0	860	26.9	1	260	123
Lot VIII	1.0	25	43.8	1,210	27.7	18	227	197
Lot IX	.3	50	33.1	920	27.7	27	204	175
Lot X	.1	34	30.6	870	28.5	21	212	164
Lot XI	.03	41	35.2	1,000	28.3	25	200	184

[1] Rounded to the nearest 10.

sac-fry was discernibly higher in lots II, III, and VIII than in lots I, IV, V, VI, IX, X and XI. Only five eggs hatched in lot VII, and these fry died within a few days. Very few fry from lot VIII survived until the feeding stage; sac-fry mortality in lots II and III was not as high. Once the fry began to feed, mortality was low in all of the lots, including the few survivors of lot VIII. The growth rates for individual collections of fry were variable but there was no apparent correlation with the exposures their parents had received. Residue data are presented in table 11.

TABLE 14.—*Mortality rates of eggs*

[Percentages are based on eggs remaining after previous pickoff]

Lot	Treatment of adults	Initial volume of eggs	Mortality after hardening	Mortality between initial pickoff and eyeing	Mortality between eyeing and hatching
		Milliliters	*Percent*	*Percent*	*Percent*
I	0	1,381	3.3	53.1	10.0
	P.p.m.				
II	1.0	1,861	.7	48.7	10.5
III	.3	1,411	1.8	40.2	13.6
IV	.1	1,360	.8	42.0	9.4
V	.03	1,553	2.0	32.9	9.5
VI	.01	1,370	2.9	34.4	11.0
	Mg./kg.				
VII	3.0	32	19.3	90+	90+
VIII	1.0	1,095	3.1	47.6	8.5
IX	.3	1,653	2.5	41.8	11.0
X	.1	1,060	.9	41.4	19.1
XI	.03	1,445	1.9	38.5	9.4

DISCUSSION

There was no indication that DDT at the levels administered in this experiment affected the rate of sexual development or gamete production of the treated adults. Fertilized eggs from treated adults developed as quickly as those from the controls, and their survival to hatching was even somewhat better. However, there was a critical period shortly after hatching when mortality was noticeably higher in offspring of the high-dosage lots. Losses were heavy in all lots at this time. Therefore, excessive mortality in some groups might indicate a lower resistance to stress. On the other hand, these high losses could conceivably be due to absorption of yolk material containing high residue levels of DDT and its metabolites.

The residue data for eggs and fry showed that appreciable amounts of chlorinated hydrocarbon are passed along by the parent fish. The results were highly variable (in one case, almost a tenfold difference existed in residue of eggs collected from two sublots receiving the same treatment), but in general, higher residues were found in the eggs of fish receiving higher dosages of DDT. The relative amounts of DDT, DDE, and DDD did not appear to differ from those in adult fish whole-body residues. No

significant pattern could be distinguished in residue levels of nonviable eggs as compared to those of unfertilized eggs, which presumably consisted of a mixture of the nonviable and viable. Residues in yolk-sac fry were somewhat higher than those in eggs from comparable lots; since no additional DDT was provided during development, this apparent increase probably resulted from variation in the samples analyzed. The lower concentrations present in later fry samples may represent a selection of a population with low residues or might result from metabolism or excretion of the chlorinated hydrocarbons.

By the time the fry were ready to accept food the daily mortality was substantially the same in all lots.

Survivors of all lots displayed equal growth and vigor during an additional 6 months of observation. However, in the case of the high-dosage lots, these survivors represented only a small portion of the reproductive potential of their parental stock when compared with the survival rate of the control progeny.

RESISTANCE TO STRESS

METHODS

An attempt was made to determine whether exposure to DDT reduced resistance to an experimentally imposed stress. Unfortunately, time was very short because of an impending sterilization of the raceways. Investigation showed that, with the available equipment, conditions could be controlled best in an experiment designed to test the ability of treated fish to withstand conditions of hyperosmolarity. The following procedures were adopted after preliminary tests to determine concentrations and exposure periods.

Test fish were taken from lots randomly, but injured or weak individuals were discarded. Treatment was carried out on approximately 20 fish at a time (14 minimum, 21 maximum) in 100 liters of aerated water containing 4 percent NaCl by weight. The temperature was maintained at 50° F., and dissolved oxygen was kept at saturation level throughout the treatment period. The fish were held in the saline solution for 120 minutes and were then released immediately into fresh water. Observation was made of the number of dead or inactive fish at 0, 10, and 30 minutes after release. A final count of dead fish was made 18 and 24 hours later when all survivors appeared to be fully recovered.

RESULTS

The combined results of these tests are shown in table 15. Fish from lots II and VIII suffered a little less mortality than the control, but these differences were not significant. The reaction of lot XI was almost identical to that in the control. However, the other treated lots lost considerably more fish.

TABLE 15.—*Distress and mortality among fish exposed to a 4-percent solution of NaCl for 2 hours*

	Number of fish	Average weight of fish (grams)	Percent of fish dead or inactive after release			Percent mortality after 24 hours	Average weight of dead fish (grams)
			At 0 minutes	At 10 minutes	At 30 minutes		
Lot I	80	205	58	42	49	30	190
Lot II	20	265	75	65	70	25	260
Lot III	20	275	100	95	90	70	280
Lot IV	40	230	92	80	80	53	240
Lot V	40	225	95	92	88	72	220
Lot VI	40	240	82	72	75	58	235
Lot VIII	14	260	57	43	43	21	165
Lot IX	60	215	98	92	92	82	215
Lot X	41	220	88	90	88	80	215
Lot XI	61	230	57	59	61	31	195

DISCUSSION

The results were inconclusive and data from these tests have been presented primarily for possible comparison with other work. The resistance shown by lots II and VIII may reflect superior physical condition of the few fish remaining in these lots. This does not explain a lower resistance in lot III which had previously displayed reactions almost identical to those of lot II. A relation between resistance to this form of stress and DDT treatment may be indicated by the higher-than-control mortality shown by six of the seven treated lots, excluding lots II and VIII.

PATHOLOGY

Examination of tissues from fish of all lots was made for evidence of pathology. Tissue changes due to parasitism were apparent throughout the experiment, but no pathology due to exposure to DDT was found.

Hematocrit measurements were routinely made on fish from all lots; no differences between treated and untreated fish were revealed. The hemoglobin decline reported in mammals was not reflected in hematocrit measurements on these trout exposed to DDT.

SUMMATION AND COMMENTS

The results of this study offer evidence of a threshold level of effectiveness of DDT in chronic poisoning of cutthroat trout. Low dosages of DDT, both in bath and in food, did not appear to affect death rate, growth, or reproductive success over an exposure period of about 20 months. The intermediate dosages given lots IV and IX had variable effects. Lot IV, given 0.1 p.p.m. DDT monthly in bath form, exhibited significantly higher mortality, similar size, and similar reproductive success when compared with the control group. Lot IX, treated once weekly with DDT in the diet at the rate of 0.3 milligrams per kilogram of body weight, did not differ from the control except, in a highly variable manner, residue buildup, and a nonsignificant increase in mortality during the last few months of the experiment. The higher dosages produced a statistically highly significant increased rate of mortality. This was the prime effect; the increase in size was a possible indirect effect based on high mortality of small, diseased, slow-growing fish. No direct effect on reproduction was observed in these lots, IV and IX, except an increased mortality of sac-fry which could not be quantitated; the reproductive potential of these fish was reduced both by this effect and by the high rates of mortality of the adult fish.

In part, at least, the threshold-type effect may be due to the limited ability of the fish to detoxify DDT. In almost every fish analyzed, three component chlorinated hydrocarbons were found: DDT, DDE, and DDD. The percentage of whole-body residues present as each of these components is presented in table 12. It can be seen that DDT ranged from about 90 percent in the higher fed groups to a low of 4 percent in the lowest fed group after one treatment. The lower-dosage-treated fish, although not different from controls in total residues, were higher in the relative amounts of DDT present and lower in DDE content. The basic diet, presumably the source of residues in the control fish, contained a chlorinated hydrocarbon mixture of about 60 percent DDE, 20 percent DDT, and 20 percent DDD. The residues in control fish were divided about 74 percent DDE, 16 percent DDT, and 9 percent DDD

(average of all controls), indicating a higher rate of metabolism and/or excretion of the latter two components.

Enzymatic dehydrochlorination of DDT to DDE has been reported in insects (Perry and Hoskins, 1950). An enzyme capable of catalyzing this reaction in vitro has been isolated and purified (Lipke and Kearns, 1960). The enzyme does not act upon DDE, but will dehydrochlorinate DDD at approximately four times the initial rate of DDT. The in vitro formation of DDE from DDT has apparently not been reported using systems obtained from higher animals, but DDE has been found in human fat (Pearce et al., 1952) to the extent of 33 to 78 percent of the total chlorinated hydrocarbons. Mattson et al. (1953) reported it present in human fat to the extent of 39 to 86 percent and Hayes et al. (1958) to the extent of 33 to 90 percent of the total. The latter group also reported that smaller proportions of DDE were present in samples obtained from humans with the greatest exposure to DDT, a finding confirmed by the present work.

Little has been reported on the metabolic fate of DDT in higher animals. A water-soluble excretory product, 2,2-bis (p-chlorophenyl)acetic acid (DDA), has been found in the urine of DDT-treated rabbits (White and Sweeney, 1945), dogs (Ofner et al., 1945), and humans (Neal et al., 1946). Burns et al. (1957) found DDA or similar compounds to be the major excretory metabolite of DDT in the rat. Excretion was primarily through the bile and feces; urinary excretion assumed importance only when the bile duct was ligated. The presence of DDD in rat tissues as a metabolite of DDT, but not of DDE, has been reported by Peterson and Robison (in press). In our laboratory, DDD has been found inconsistently in fish tissues after field exposure to commercial preparations of DDT. The concentrations present in whole bodies of trout in this study probably do not represent accumulation by metabolic formation from DDT. There was a downward trend in the percentage of DDD present in these fish as the study progressed, suggesting that levels of DDT and DDE were building up at a faster rate than DDD. In another experiment (Bridges, Kallman, and Andrews, 1963), in which a warm-water pond was treated once with p,p′-DDT, we have found DDD present in fish and crayfish in increasing proportion since about 1 week after the treatment. After 14 months, DDD was present to the extent of 73 percent of the total chlorinated hydrocarbons in rainbow trout. Its identity was established by chromatography in two solvent systems, using known DDD as reference, and by passage through a column of MgO, producing a dehydrochlorinated derivative which was chromatographically identical to that of known DDD.

Analysis of tissue and organ pools obtained from fish which died during the experiment indicated a highly uneven distribution of total chlorinated hydrocarbons and of the three components. Distribution of the components seems not to be a function of the lipid content of the various organs. Even in those organs with high DDD contents, sufficient DDD was present in the basic diet to account for the total, assuming selective retention of of this substance by the organ. McKinley and Grice (1960) found that both DDD and DDT are retained in tissues to a greater extent than are similar substances. However, Finnegan et al. (1949), reported that in dogs DDD is both metabolized to DDA and excreted in the feces to a greater extent than DDT. As mentioned above, there is no evidence from our whole-body residue data that DDD was selectively retained in the control fish of the study.

No clear pattern of relative distribution emerges from the residue data of tissue pools. Brain, for example, was only

slightly higher than ovary in lot II, but contained more than fivefold the residue of ovary in lot VIII. In the low-dosage pools, ovary contained higher residues than brain. Residues in muscle and liver were similar to each other in lots II and VIII, but with markedly different relative amounts of the three component chlorinated hydrocarbons. Muscle in all pools contained a high proportion of DDT, while liver was uniformly low in this component. Total residues in testes were low in three pools, but were the highest in the fourth.

Comparison of these results with the findings of Holden (1962) are of interest. Holden exposed brown trout to acutely lethal dosages of C^{14}-DDT in water. The calculated tissue concentrations, based on radioactivity and expressed as DDT, were generally higher in liver and muscle than in brain. The carbon tetrachloride-extractable material expressed as a percentage of the organ weight was higher in brain than in liver and muscle. A direct relation between the concentration of material extractable by this solvent and the DDT concentration in the organ did not exist after 16 hours of exposure. After 160 or 230 hours, such a relation did exist, except in brain. Brain was consistently high in "lipid" and low in DDT in this short-term study. At 16 hours, gill filaments were lowest in "lipid" and highest in DDT content. At 160 hours they were also lowest in DDT.

The question of the cause of death of fish with a given residue of a chlorinated hydrocarbon insecticide is often of importance from a management or forensic standpoint. Whole-body residues of total chlorinated hydrocarbons, however high, are insufficient data in most cases, especially when the symptoms exhibited by the fish either were not observed or were not typical of this type of poisoning. Often fish with extremely high residues appear normal; fish taken from Clear Lake, Calif., some time after an application of DDD had residues of over 2,000 p.p.m. in fat, but were in apparent good health (Hunt and Bischoff, 1960).

With the chlorinated hydrocarbons whose metabolic pathways are known in at least some detail, both the total residue and its distribution in terms of toxicant and metabolites should be of value in deciding upon the cause of death. In our work, fish treated with the highest levels of DDT exhibited early marked mortality and had smaller relative amounts of the metabolite DDE present. Although mortality was probably not caused directly by DDT, but rather by other stresses, it can be related to both total residue and extent of metabolism. Heaviest mortalities in these lots occurred before the maximum relative amounts of DDT were reached. This was perhaps a result of a population change; the fish which succumbed were not routinely analyzed for total body residues, but may have had higher relative DDT concentrations than survivors.

The high-dosage lots of this experiment may be considered as representing a state between acute and chronic exposure to DDT since heavy mortality was observed early in the study and occurred at a rapid rate. In the more truly chronic aspects, in the low-treatment groups (lots V, VI, X, and XI), there was an increased relative amount of DDT present, but neither mortality nor total residues differed significantly from lot I. The percentage of DDT present in fish of lots IV and IX gradually increased with increasing residues to a level intermediate to the high- and low-dosage lots. Mortality was high only in lot IV, and in this lot the increased mortality occurred both early in the study, when relative DDT content was higher than that of low-dosage lots, and later, when there was probably no significant difference in relative amounts of DDT and DDE between lots IV and V, but

when total residues were higher in lot IV. In long-term exposures involving repeated dosages of DDT, mortality probably cannot be related to a "metabolic index" such as [DDE]/[DDT]. This may be due in part to a chronic effect of DDE, whose toxicity to fish has not as yet been determined.

Residue levels in individual organs may offer another important criterion in judging cause of death. The brain appears particularly promising in this respect, although further work must be done with acutely lethal doses of DDT. Brains of fish of lots II and VIII that died had total residues far higher than whole-body residues of living fish sampled at comparable times. This was not true of brains of fish which died spontaneously in lots V–VI and X–XI, in which mortality differed little from control levels.

At our present state of knowledge, the best means of assessing cause of death in fish exposed to DDT appears to be by judgment of the combination of factors: Total residue, metabolic index, and residue in the brain. No absolute "lethal levels" are known for any of these factors, and wide latitudes must be allowed in any given situation. Needless to say, a knowledge of the history of pesticide use prior to any fish kill is important.

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